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Reanalysis of PFO5DoA Levels in Blood from Wilmington, North Carolina, Residents, 2017–2018

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Introduction

Perfluoro-3,5,7,9,11-pentaoxadodecanoic acid (PFO5DoA, DTXSID50723994) is a perfluoroalkyl ether acid (PFEA) produced at a fluorochemical facility ("Fayetteville Works") in Bladen County, North Carolina. In 2015, PFO5DoA was first identified in Cape Fear River water samples collected downstream of the facility's wastewater discharge point.[1] Approximately 280,000 people rely on public water sourced from the lower Cape Fear River.[2] The GenX Exposure Study started in 2017 to characterize PFEA exposure in Cape Fear River Basin, North Carolina, residents. We detected three PFEAs—ethanesulfonic acid, 2-[1-[difluoro(1,2,2,2-tetrafluoroethoxy)methyl]-1,2,2,2-tetrafluoroethoxy]-1,1,2,2-tetrafluoro- (also known as Nafion by-product 2, DTXSID10892352); perfluoro (3,5,7,9-butaoxadecanoic) acid (PFO4DA, DTXSID90723993); and PFO5DoA—in blood serum from nearly all 344 participants who resided in Wilmington, North Carolina, and provided blood samples in 2017 and 2018.[3]

In 2018, serum samples were analyzed by liquid chromatography coupled to high-resolution mass spectrometry (LC-HRMS). At the time, an analytical standard for PFO5DoA was not commercially available and we were unaware of other laboratories analyzing serum for PFO5DoA, which limited interlaboratory comparison opportunities. We have since discovered a mass interference in the calibration of our PFO5DoA analysis that resulted in substantial underestimation of PFO5DoA concentrations; the other per- and polyfluoroalkyl substances (PFAS) values were not affected. This letter aims to correct previously reported serum PFO5DoA concentrations.[3]

Methods

Sample Selection

In 2018, we analyzed 388 serum samples from 344 participants (44 participants provided two samples) for 20 PFAS, including PFO5DoA, across eight analytical batches,[3] with 30 to 61 samples per batch, but most batches had ~50 samples. The PFO5DoA calibration curve without the mass interference had poor linearity. Because of the time lag between the original analysis and calibration error discovery, application of a new PFO5DoA calibration curve to the original response ratios would have resulted in concentrations with substantial uncertainty. We chose to reanalyze a subset of the 388 serum samples for PFO5DoA and use the reanalysis results to predict corrected concentrations for the remaining samples that were not reanalyzed. To select samples for reanalysis, we computed batch-specific summary statistics for PFO5DoA concentration among the samples with detectable concentrations.[3] We randomly selected one sample within each octile for each batch to provide data for calibration across the full range of concentrations and randomly selected two samples with non-detectable levels per batch to get a total of 80 samples for reanalysis.

PFO5DoA Reanalysis

An analytical standard for PFO5DoA was acquired from Fluoryx Labs (Catalog no. FC23-PFO5DOANA). Perfluoro-*n*-[$^{13}C_8$]octanoic acid (Catalog no. CLM-8005-1.2; Cambridge Isotope Laboratories) was used as an internal standard for PFO5DoA quantitation. A 50-μL aliquot of serum was mixed with 150 μL of cold methanol containing internal standards (1.00 ng/mL final concentration). The mixture was vortexed and centrifuged at $10{,}000 \times g$ for 5 min. A 100-μL aliquot of supernatant was mixed with 50 μL of water to produce a final sample containing 50% methanol by volume. We used a fluorinated column (Kinetex F5, 2.6 μm particle, 100×2.1 mm analytical column; part number 00D-4723-AN) from Phenomenex on a Thermo Scientific Vanquish LC coupled to an Orbitrap Exploris 240.[4] The method reporting limit (MRL) of 0.5 ng/mL PFO5DoA corresponded to the lowest calibration standard which was within 30% of the true value.

Statistical Methods

The mass interference impacted the PFO5DoA calibration curve but not the PFO5DoA mass spectrometer response for the samples, in which the unintended mass was absent. Therefore, we kept the response ratios [ratio of PFO5DoA response to mass-labeled perfluorooctanoic acid (PFOA) response] from the original analysis in 2018 for all 388 samples. We also had PFO5DoA concentrations for the 80 samples reanalyzed in 2022. The model that best fit the data based on standard residual diagnostics, parsimony, and stability through the range of 388 response ratios from 2018, was a two-part, piecewise, weighted least squares linear regression model. The model was weighted by the inverse square root of the 2018 response ratio to account for heteroskedasticity. The model was fit without an intercept so that a 2018 instrument response of 0 (which occurred for three samples) predicted a concentration of 0 ng/mL. Leave-one-out cross-validation was used to assess the model's goodness of fit; to get the predicted value for each reanalyzed observation, that reanalyzed observation was

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The authors declare they have nothing to disclose.

Table 1. Summary statistics for corrected PFO5DoA concentrations in first serum sample from 344 Wilmington, North Carolina, residents in 2017–2018.

				PFO5DoA concentration (ng/mL)				
Category	Group	n	$n >$ MRL[a] (%)	10th percentile	25th percentile	Median	75th percentile	95th percentile
Reanalyzed and predicted[b]	Adults	289	285 (99)	3.5	5.6	10.1	16.3	28.7
	Children	55	54 (98)	3.2	4.5	5.7	9.1	12.4
	All	344	339 (99)	3.4	5.2	9.2	14.8	26.6
Reanalyzed only[c]	All	80	80 (100)	2.3	4.5	8.7	14.8	25.5

Note: MRL, method reporting limit; PFO5DoA, perfluoro-3,5,7,9,11-pentaoxadodecanoic acid.
[a]The MRL for the PFO5DoA reanalysis was 0.5 ng/mL.
[b]Corrected concentrations for the 344 serum samples are based on 72 reanalyzed sample concentrations and 272 sample concentrations predicted by regression modeling.
[c]Concentrations for 80 reanalyzed samples used to build regression model. Eight of the 80 participants whose samples were reanalyzed were repeaters; they had provided two blood samples, and we reanalyzed their second sample but not their first. Thus, 72 reanalyzed sample concentrations were included in summary statistics for first serum sample.

removed from the dataset. The reduced dataset was used to obtain the prediction for that observation according to the model.

Because the model aimed to predict sample concentrations as if all samples were rerun in 2022, we applied the 2022 analytical MRL (0.5 ng/mL) to determine detection frequency. We used Spearman's correlation to assess correlation between incorrect 2018 concentration and corrected 2022 concentration. For summary statistics calculation, values <MRL were assigned a value of $\frac{0.5}{\sqrt{2}}$ (~ 0.354 ng/mL). We estimated a bootstrap standard error (SE) for the PFO5DoA median by resampling model residuals. Residuals of the original regression model were unweighted, resampled with replacement, reweighted, and then added to the original fitted values in each bootstrap iteration to obtain a resampled dataset. Other statistical analyses (i.e., calculation of summed PFAS concentration in serum and percent change in PFAS concentration from November 2017 to May 2018 for repeaters) followed our previous methods.[3]

All phases of the study were conducted in compliance with the North Carolina State University institutional review board.

Results and Discussion

The final dataset contained 80 reanalyzed and 308 predicted concentrations for the 388 samples. For the 80 samples reanalyzed by LC-HRMS, predictions of the corrected concentrations were strongly correlated with the concentrations determined by reanalysis ($r_s = 0.94$). The strong cross-validated correlation and the fact that the samples were selected to be representative of the 388-sample set suggested that predicted concentrations could serve as a reasonable replacement for reanalyzing all samples. Ultimately, the corrected concentrations for the 388-sample set were strongly correlated with the 2018 (incorrect) concentrations ($r_s = 0.98$), suggesting that the sample rank order was largely preserved.

Summary statistics for corrected PFO5DoA concentrations are shown for 344 GenX Exposure Study participants in Wilmington in 2017–2018 (Table 1). PFO5DoA was detected in all 80 reanalyzed samples and, after applying the predictive model, concentrations exceeded the MRL in 339 of 344 participants (99%). The median serum PFO5DoA concentration (9.2 ng/mL, bootstrap SE = 0.35 ng/mL) was much higher than previously reported (0.3 ng/mL).[3] The median percent decrease in serum PFO5DoA levels from November 2017 to May 2018 across 44 participants was 27.4% [95% confidence interval (CI) = 18.3%, 36.5%]. In addition, serum PFO5DoA levels in participants served with treated Cape Fear River water ($n = 333$) were significantly higher (median = 9.3 ng/mL; range = ND, 51 ng/mL) than levels in participants served with another water source ($n = 9$, median = 3.4, range = ND, 6.5 ng/mL) ($p = 0.0002$).

Taking the corrected PFO5DoA values with our previous results for other PFEAs, median serum concentration in the Wilmington 2017–2018 population increased with increasing number of $-CF_2O-$ groups in the chemical structure [i.e., PFO3OA (median <MRL) <PFO4DA (median = 2.5 ng/mL) <PFO5DoA (median = 9.2 ng/mL)]. PFO5DoA had the highest median concentration of the PFAS quantified and contributed substantially to the summed concentration of targeted PFAS in Wilmington serum samples (Table 2). PFO5DoA concentrations were similar to perfluorooctanesulfonic acid (PFOS) concentrations (median = 8.6 ng/mL; IQR = 5, 13.6 ng/mL). PFO5DoA and PFOS each contributed ~30% to the summed PFAS concentration in serum; the next highest contributor was PFOA (~10%). For November 2017

Table 2. Summed mass concentrations of PFEAs (PFO3OA, PFO4DA, PFO5DoA, NVHOS, Nafion by-product 2) and legacy PFAS (PFHpA, PFOA, PFNA, PFHxS, PFOS) in serum from 344 Wilmington, North Carolina, residents, 2017–2018.

	Concentration [ng/mL (percentage of total PFAS)][a]				
Category	10th percentile	25th percentile	Median	75th percentile	95th percentile
∑ PFEAs[b]					
Adults	5.3 (36)	8.9 (37)	16.2 (43)	27.6 (47)	46.7 (52)
Children	4.7 (40)	7.2 (41)	10.7 (46)	17.6 (55)	24.4 (51)
Overall	5.2 (37)	8.5 (40)	15.3 (45)	25.1 (46)	45.5 (53)
∑ legacy PFAS					
Adults	8 (54)	12.2 (51)	20.8 (55)	29.8 (51)	47.8 (54)
Children	6.8 (58)	8.1 (47)	11.3 (48)	16.4 (51)	24 (50)
Overall	7.6 (54)	11.1 (52)	18.8 (55)	28.7 (53)	47.1 (55)
∑ all PFAS					
Adults	14.9	23.9	37.9	58.6	89.4
Children	11.7	17.4	23.4	31.9	47.9
Overall	14.2	21.5	34.3	54.6	85.9

Note: Nafion by-product 2, ethanesulfonic acid, 2-[1-[difluoro(1,2,2,2-tetrafluoroethoxy)methyl]-1,2,2,2-tetrafluoroethoxy]-1,1,2,2-tetrafluoro-; NVHOS, 1,1,2,2-tetrafluoro-2-(1,2,2,2-tetrafluoro-ethoxy)ethane sulfonate; PFAS, per- and polyfluoroalkyl substances; PFEA, per- and polyfluoroalkyl ether acid; PFHpA, perfluoroheptanoic acid; PFHxS, perfluorohexanesulfonic acid; PFNA, perfluorononanoic acid; PFO3OA, perfluoro-3,5,7-trioxaoctanoic acid; PFO4DA, perfluoro-3,5,7,9-butaoxadecanoic acid; PFO5DoA, perfluoro-3,5,7,9,11-pentaoxadodecanoic acid; PFOA, perfluorooctanoic acid; PFOS, perfluorooctanesulfonic acid.
[a]Percentage of total PFAS concentration (the sum of PFEAs and legacy PFAS analyzed for in this study) is shown in parentheses.
[b]The PFEA term is synonymous with "fluoroethers," which is the term we used in our previous publication.[3]

participants ($n = 310$), corrected PFO5DoA concentrations were highly correlated with concentrations of Nafion by-product 2 ($r_s = 0.87$), perfluorohexanesulfonic acid (PFHxS; $r_s = 0.73$), PFOA ($r_s = 0.8$), and perfluorononanoic acid (PFNA; $r_s = 0.71$). In the time since we reported detecting PFO5DoA in Wilmington residents' serum, others have reported on PFO5DoA-exposed populations[5–7] and results of animal studies of PFO5DoA toxicity.[8] Further investigation of PFO5DoA exposure and potential health effects in Cape Fear River Basin residents[9] is needed.

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